S/N 08/955,572 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Byoung S. Kwon	Examiner:Unknown
Serial No.:	cont. of 08/955,572	Group Art Unit: Unknown
Filed:	Herewith	Docket: 740.013US3
Title:	NEW RECEPTOR AND RELATED PRODUCTS AND METHODS	

PRELIMINARY AMENDMENT

Box Patent Application
Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above-identified continuation application as follows.

In the Specification

Please enter the enclosed SEQUENCE LISTING into the specification.

Please make the paragraph substitutions indicated in the appendix entitled "Clean Version of Amended Specification Paragraphs." The specific changes incorporated in the substitute paragraphs are shown in the following marked-up versions of the original paragraphs.

The paragraph beginning on page 1, line 6, is amended as follows:

This application is a continuation of U.S. application Serial No. 08/955,572, filed October 22, 1997, currently pending, which is a file wrapper continuation of U.S. application Serial No. 08/461,652, filed June 5, 1995, which is a division of U.S. application Serial No. 08/122,796, filed September 16, 1993, abandoned [continuation-in-part of copending application Serial No. 08/012,269, filed February 1, 1993, which is a continuation-in-part of copending application Serial No. 07/922,996 filed July 30, 1992, which is a continuation-in-part of copending application Serial No. 07/267,577 filed November 7, 1988].

The paragraph beginning on page 6, line 16, is amended as follows:

Still another object of the present invention is to teach methods of using the cDNA H4-1BB, the receptor protein H4-1BB, the monoclonal antibody and the [legand] ligand for H4-1BB.

The paragraph beginning on page 6, line 24, is amended as follows:

[Figure 1 shows] Figures 1A and 1B show the nucleotide sequence for the cDNA (SEQ ID NO:9) of mouse receptor protein 4-1BB, which encodes a polypeptide having SEQ ID NO:10, and the regions used as PCR primers to obtain the human homologue H4-1BB.

The paragraph beginning on page 7, line 11, is amended as follows:

[Figure 1 shows] Figures 1A and 1B show the nucleotide sequence and the deduced amino acid sequence of the mouse receptor 4-1BB also shown in SEQ ID NO:9 and SEQ ID NO:10. The nucleotides of the message strand are numbered in the 5' to 3' direction and numbers are shown on both sides of the sequence. Nucleotide residue 1 is the A of the initiation codon ATG, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers. The predicted amino acid sequence is shown below the nucleotide sequence. Putative signal peptide is underlined. Stop codon is indicated by (---). Cysteine residues are highlighted by the dots. An unusual feature of 4-1BB sequence is that there is a potential polyadenylation signal of AATAAA at nucleotides 1158-1163 [(Fig. 1 boxed)] (Fig. 1B boxed). It was believed that this signal was functional because this gene produces at least two different sizes of mRNA.

The paragraph beginning on page 8, line 29, continuing to page 9, line 18, is amended as follows:

4-1BB is structurally related to members of the nerve growth factor receptor super-family. Although these receptors possess structurally similar ligand-binding properties (cysteine-rich regions), the cytoplasmic domains of these proteins are nonconserved which could allow for diversity in transmembrane signaling. Some members of this family are involved in the T or B cell activation process. There are in vitro functional data on the OX-40, CD40 and CD27 antigens. Antibodies against the OX-40 augment the T cell response in a mixed lymphocyte cell response in a mixed lymphocyte reaction (7) and antibodies against CD40 B-cell proliferation in the presence of a coactivator, such as PMA or CD20 antibodies, and synergize with IL-4 in vitro to induce B-cell differentiation and to generate long-term normal B cell lines (8). One [inonoclonal] monoclonal antibody, anti-1A4, which recognizes an epitope on the CD27

molecule inhibited calcium mobilization, IL-2 secretion, helper T cell function, and T cell proliferation. On the other hand, CLB-CD27/1, another anti-CD27 mAb enhanced proiferation of human T cells stimulated with PHA or anti-CD3 mAb (6). These results indicate that the CD27 molecule plays an important role in T cell activation. Except for TNFRs, NCFR and CD40, the ligands or cell surface molecules to which the members of the superfamily bind are not yet identified. Identification and characterization of the ligands to which the receptors bind will be helpful in better defining the physiologic role of 4-1BB.

The paragraph beginning on page 11, line 3, is amended as follows:

This region forms the pattern of C-X2-C-X9-C-X3-H-X3-C-X-C (SEQ ID NO:11); and the cysteines and histidine are conserved in a similar space in 4-1BB, *sina*, and DG17 proteins. Ten of 24 amino acids between the 4-1BB and *sina* proteins are identical, and 3 of 24, are conservative substitutes. The conserved pattern suggests that these amino acids are functionally important. The *sina* protein is localized in the nucleus, suggesting that it has a regulatory function in cells. The fact that the amino acid sequence of 4-1BB contains features like a zinc finger motif, a nuclear protein, and a receptor domain suggests that 4-1BB may play diverse roles during cellular proliferation and differentiation.

The paragraph beginning on page 13, line 20, and continuing on page 14, is amended as follows:

Another member of the NGFR superfamily, CD40, is expressed on B cells and interacts with gp39, a molecule expressed on activated T cells. The cDNAs encoding the murine (29) and human (30) gp39 proteins have been cloned; this cell surface molecule is a type II membrane protein with homology to tumor necrosis factor. Noelle et al. (31) found that a CD40-[inununoglobulin] immunoglobulin fusion protein, is capable of blocking T cell-induced B-cell proliferation and differentiation in a dose-dependent manner. Armitage et al. have isolated a cDNA for murine gp39 and showed that gp39 could induce B-cell proliferation in the absence of co-stimuli, and result in IgE production in the presence of [IL-4-] IL-4. Hollenbaugh et al. (32) have shown that COS cells transfected with human [gp 39] gp39 can synergize with either TPA

or anti-CD20 in inducing human B-cell proliferation and is able to stimulate B cells without a costimulator only at low levels. These data indicate that CD40 may be one of the B-cell-surface molecules that transmit signals during physical contact with T cells.

The paragraph beginning on page 14, line 13, is amended as follows:

In addition, one ligand may function as both a cell surface and soluble ligand. Recent evidence on the [CD4-0] CD40 ligand, gp39, suggests that this ligand can exist as a membrane bound as well as a soluble ligand (35). It may be possible that 4-1BB is secreted and interacts with B cells in a soluble form as well as a membrane bound form. A member of the NGFR receptor family, CD27, which is expressed on T cells, is secreted in addition to being expressed on the cell surface (36). It is also possible that more than one 1 ligand (soluble and cell surface) may bind to 4-1BB.

The paragraph beginning on page 14, line 36 and continuing to page 15, line 5, is amended as follows:

Forward primer I (H4-1BBFII) spans from amino acids 36 to 41 and forward primer II (HR-1BBFII) spans from amino acids 52 to 58 of the mouse 4-1BB. Reverse primer I (H4-1BBRI) spans from amino acids 116 to 121 and reverse primer II (H4-1BBRII) spans from amino acids 122 to 128 of mouse 4-1BB. The regions used as PCR primers in mouse 4-1BB are indicated [if Fig. 1] in Figure 1A.

The paragraph beginning on page 15, line 6, is amended as follows:

The [degenative] degenerate oligonucleotide sequence of each primer is as follows:

The paragraph beginning on page 15, line 8, is amended as follows:

SEQ ID NO:3/H4-1BBFI: 5' TTC TGT CGI AAA TAT AAT CC 3'

The paragraph beginning on page 15, line 11 is amended as follows:

SEQ ID NO:4/H4-1BBRI: 5' TTC TCI TCI ATT GGI GGI CA 3'

The paragraph beginning on page 15, line 15 is amended as follows:

SEQ ID NO:6/H4-1BBRI: 5' CC IAA IGA ACA IGT TTT ACA 3'

The paragraph beginning on page 15, line 18 is amended as follows:

SEQ ID NO:7/H4-1BBRII: 5' TT TTG ATC ATT AAA IGT ICC 3'

The paragraph beginning on page 15, line 30 and continuing to page 16, line 2 is amended as follows:

The primer set of H4-1BBFII and H4-1BBRII produced a specific band of ~240bp. The 240bp is an expected size of human 4-1BB if the human homologue protein is similar to mouse 4-1BB in size. The PCR product (240bp) was cloned in PGEM3 vector and sequenced. One open reading frame of the PCR product was ~65% identical to mouse 4-1BB. Therefore, it was concluded that the 240 bp PCR product is the human homologue of mouse 4-1BB. The 240 bp PCR product was used to screen λgt11 cDNA library of activate human T lymphocytes. An ~0.85 kb cDNA was isolated. The sequence of the cDNA is shown in Figure 2 and the predicted amino acid sequence is shown in Figure 2b. The same information is shown is the sequence listing attached to this specification in [sequence id. 1] SEQ. ID NO:1.

The paragraph beginning on page 17, line 37, and continuing on page 38, is amended as follows:

As illustrated with mouse receptor 4-1BB and mouse [ligan] ligand 4-1BBL above, addition of H4-1BB-AP will coat the H4-1BB expressing cells and block the normal interaction between H4-1BB and H4-1BBL. This will lead to immunosuppression. This type of immunosuppression is antigen-specific. Therefore it avoids the generalized immunosuppression produced by antiCD3 or cyclosporin A treatments. H4-1BB-AP treatment can be used to treat certain autoimmune diseases and to facilitate organ transplantation.

In the Claims

Please substitute the claim set entitled "Clean Version of Pending Claims" for the pending claim set. Specific amendments to the claims are detailed in the following marked-up set of claims.

Please cancel claims 5-18 without prejudice.

Please amend the claims as follows:

1. (Amended) [A cDNA] An isolated nucleic acid molecule encoding for a human receptor protein H4-1BB comprising SEQ ID NO:2.

2. (Amended) The [cDNA] isolated nucleic acid molecule of claim 1 having a nucleotide sequence [as shown in Figure 2] comprising SEQ ID NO:1.

3. (Amended) The [cDNA] isolated nucleic acid molecule of claim 1, identified as pH4-1BB deposited at the Agricultural Research Service Culture Collection with the accession number NRRL B21131.

4. (Amended) The [cDNA] isolated nucleic acid molecule of claim 2 and fragments [and derivatives] thereof, wherein said fragments [and derivatives can be used as a probed to isolate DNA sequences encoding for proteins similar to the receptor protein encoded by said cDNA] encode a protein comprising the extracellular domain of SEQ ID NO:2.

Please add the following new claims:

19. (New) An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) nucleotides of H4-1BBFI (SEQ ID NO:3);

b) nucleotides of H4-1BBFII (SEQ ID NO:4);

c) nucleotides of H4-1BBRI (SEQ ID NO:5);

d) nucleotides of H4-1BBRII (SEQ ID NO:6);

e) nucleotides of SEQ ID NO:7;

f) nucleotides of SEQ ID NO:8;

g) nucleotides of a DNA encoding a soluble human 4-1BB polypeptide comprising the extracellular domain of human 4-1BB (amino acids 1-186 of SEQ ID NO:2) or a fragment of the extracellular domain capable of binding a 4-1BB-L; and

h) nucleotides of subparagraph g) wherein said DNA additionally encodes a polypeptide that is not a human 4-1BB polypeptide and which is located C-terminal to the extracellular domain of human 4-1BB polypeptide.

20. (New) An isolated nucleic acid molecule encoding a human 4-1BB polypeptide, wherein said molecule comprises a nucleotide sequence selected from the group consisting of:

a) nucleotides 41-805 of SEQ ID NO:1; and

b) nucleotides 41-598 of SEQ ID NO:1.

21. (New) A recombinant expression vector comprising the nucleic acid molecule of claim 19 g), 19 h) or 20 operably linked to regulatory sequences suitable for expression of the nucleic acid molecule in a host cell.

22. (New) A recombinant expression vector comprising a recombinant nucleic acid molecule comprising a nucleic acid segment encoding SEQ ID NO:2 or the extracellular domain thereof operably linked to regulatory sequences suitable for expression of the nucleic acid segment in a host cell.

23. (New) The recombinant expression vector of claim 22 wherein the recombinant nucleic acid molecule further comprises a nucleic acid segment that encodes a polypeptide that is not SEQ ID NO:2 or the extracellular domain thereof and which is located C-terminal to SEQ ID NO:2 or the extracellular domain thereof.

Remarks

Claims 5-18 are canceled. Claims 1-4 are amended, and claims 19-23 are added. The pending claims are claims 5-23.

Support for the amendments to claims 1, 2 and 3 is found in Figure 2.

Support for the amendment to claim 4 is found in the specification at page 16, lines 4-22 and in Figure 2B.

Support for newly added claim 19 is found in originally-filed claims 14-15 and in the specification at page 15, lines 8-20, and page 16, lines 4-21.

Support for newly added claim 20 is found in the specification at page 16, lines 4-21, and in Figures 2a and 2b.

Support for newly added claims 21, 22 and 23 is found in claims 5 and 14-15 as originally filed, and in the specification at page 16, lines 4-10.

This Preliminary Amendment and the above-referenced SEQUENCE LISTING are filed in part to conform the above-referenced application to the requirements of 37 C.F.R. §§ 1.821 - 1.825. To conform the above-referenced application to the requirements of 37 C.F.R. §§ 1.821 through 1.825, a paper copy of a Sequence Listing is submitted herewith. The paper copy of the Sequence Listing in this application is identical to the computer readable form of the Sequence Listing filed in application Serial No. 08/955,572, filed October 22, 1997. In accordance with 37 C.F.R. § 1.821(e), please use the computer readable form filed on January 24, 2000, in application Serial No. 08/955,572, as the computer readable form for the instant application.

The Examiner is respectfully requested to enter the above-mentioned amendments prior to taking the application up for examination. If any questions remain with respect to the present application, the Examiner is requested to contact Applicant's Representatives at the below-listed number.

Respectfully submitted,

BYOUNG S. KWON,

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6959

Date December 20, 2001 By _____
Janet E. Embretson
Reg. No. 39,665

"Express Mail" mailing label number: EL873860965US
Date of Deposit: December 20, 2001
This paper or fee is being deposited on the date indicated above with the United States Postal Service pursuant to 37 CFR 1.10, and is addressed to The Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Docket No. 00740.013US3
WD # 415861.wpd

Client Reference No. Unknown

Clean Version of Pending Claims

NEW RECEPTOR AND RELATED PRODUCTS AND METHODS
Applicant: Byoung S. Kwon
Serial No.:

---

1. (Amended) An isolated nucleic acid molecule encoding for a human receptor protein H4-1BB comprising SEQ ID NO:2.

2. (Amended) The isolated nucleic acid molecule of claim 1 having a nucleotide sequence comprising SEQ ID NO:1.

3. (Amended) The isolated nucleic acid molecule of claim 1, identified as pH4-1BB deposited at the Agricultural Research Service Culture Collection with the accession number NRRL B21131.

4. (Amended) The isolated nucleic acid molecule of claim 2 and fragments thereof, wherein said fragments encode a protein comprising the extracellular domain of SEQ ID NO:2.

19. (New) An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) nucleotides of H4-1BBFI (SEQ ID NO:3);
b) nucleotides of H4-1BBFII (SEQ ID NO:4);
c) nucleotides of H4-1BBRI (SEQ ID NO:5);
d) nucleotides of H4-1BBRII (SEQ ID NO:6);
e) nucleotides of SEQ ID NO:7;
f) nucleotides of SEQ ID NO:8;
g) nucleotides of a DNA encoding a soluble human 4-1BB polypeptide comprising the extracellular domain of human 4-1BB (amino acids 1-186 of SEQ ID NO:2)

or a fragment of the extracellular domain capable of binding a 4-1BB-L; and

h) nucleotides of subparagraph g) wherein said DNA additionally encodes a polypeptide that is not a human 4-1BB polypeptide and which is located C-terminal to the extracellular domain of human 4-1BB polypeptide.

20. (New) An isolated nucleic acid molecule encoding a human 4-1BB polypeptide, wherein said molecule comprises a nucleotide sequence selected from the group consisting of:

a) nucleotides 41-805 of SEQ ID NO:1; and

b) nucleotides 41-598 of SEQ ID NO:1.

21. (New) A recombinant expression vector comprising the nucleic acid molecule of claim 19 g), 19 h) or 20 operably linked to regulatory sequences suitable for expression of the nucleic acid molecule in a host cell.

22. (New) A recombinant expression vector comprising a recombinant nucleic acid molecule comprising a nucleic acid segment encoding SEQ ID NO:2 or the extracellular domain thereof operably linked to regulatory sequences suitable for expression of the nucleic acid segment in a host cell.

23. (New) The recombinant expression vector of claim 22 wherein the recombinant nucleic acid molecule further comprises a nucleic acid segment that encodes a polypeptide that is not SEQ ID NO:2 or the extracellular domain thereof and which is located C-terminal to SEQ ID NO:2 or the extracellular domain thereof.